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A prospective study of the association between SARS-CoV-2 infection and COVID-19 vaccination with changes in usual menstrual cycle characteristics

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Title: A prospective study of the association between SARS-CoV-2 infection and COVID-19 vaccination with changes in usual menstrual cycle characteristics

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Condensation: COVID-19 vaccination, but not SARS-CoV-2 infection, was associated with 1.7-fold increased risk of a short-term (<6 months) increase in usual menstrual cycle length.

Short title: COVID-19 infection and vaccination and menstrual cycle changes

AJOG at a Glance:

A. Why was this study conducted?

Despite the increasing public questions and mistrust with regard to the potential adverse reproductive impacts of COVID-19 infection and vaccination, there is still limited research investigating this topic.

B. What are the key findings?

- In this prospective study of 3,858 pre-menopausal nurses, vaccination against COVID-19 was associated with 1.7-fold increased risk of a short-term (<6 months) increase in usual menstrual cycle length, especially among women who had irregular, short or long pre-vaccination menstrual cycles.
- These associations were observed for both mRNA and adenovirus-vectored vaccines and were not explained by pandemic-related behavioral changes and mental stress.

SARS-CoV-2 infection was not related to changes in menstrual characteristics.

C. What does this study add to what is already known?

These findings have implications for vaccine developers, clinicians, and researchers in better understanding the possible menstrual cycle changes post-vaccination and to inform patient expectations.

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Abstract

Background Despite anecdotal reports, the impacts of SARS-CoV-2 infection or COVID-19 vaccination on menstrual health have not been systemically investigated.

Objective To examine the associations of SARS-CoV-2 infection and COVID-19 vaccination with menstrual cycle characteristics.

Study Design We prospectively followed 3,858 pre-menopausal women in the Nurses' Health Study 3 (NHS3) living in the United States or Canada who received biannual follow-up questionnaires between January 2011 and December 2021, and completed additional monthly and quarterly surveys related to the COVID-19 pandemic between April 2020 and November 2021. History of positive SARS-CoV-2 test, COVID-19 vaccination status, and vaccine type were self-reported in surveys conducted in 2020 and 2021. Current menstrual cycle length and regularity "pre-COVID" (reported at baseline between 2011-2016) and "post-COVID" (reported in late 2021). Pre- to post-COVID change in menstrual cycle length and regularity was calculated between reports. Logistic or multinomial logistic regression models were used to assess the associations between (1) SARS-CoV-2 infection and (2) COVID-19 vaccination and change in menstrual cycle characteristics.

Results The median age at baseline and end of follow-up were 33 years (range=21-51) and 42 years (range=27-56), respectively, with a median follow-up time of 9.2 years. We documented 421 (10.9%) SARS-CoV-2 infections and 3,527 (91.4%) vaccinations during follow-up. Vaccinated women had a higher risk of increased cycle length compared to those unvaccinated (OR=1.54, 95% CI=1.04-2.27), after adjusting for sociodemographic and behavioral factors. These associations were similar after additionally accounting for pandemic-related stress. COVID-19 vaccination was only associated with change to longer cycles in the first 6 months after vaccination (0-6 months: OR=1.72, 95% CI=1.08-2.73; 7-9 months: OR=1.49, 95% CI=1.00-2.23; >9 months: OR=1.44, 95% CI=0.93-2.22) and among women whose cycles were short, long or irregular before vaccination (OR=2.87, 95% CI=1.54-5.35; OR=1.03, 95% CI=0.60-1.75 for women with normal length, regular cycles before vaccination). mRNA and adenovirus-vectored vaccines were both associated with this change. SARS-CoV-2 infection was not associated with changes in usual menstrual cycle characteristics.

Conclusions and Relevance COVID-19 vaccination may be associated with short-term changes in usual menstrual cycle length, particularly among women whose cycles were short, long or irregular before vaccination. These results underscore the importance of monitoring menstrual health in vaccine clinical trials. Future work should examine the potential biological mechanisms.

Key words: COVID-19 vaccine; SARS-CoV-2 infection; menstrual cycle change; menstrual cycle length; menstrual health

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Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected more than 48 million people in the United States (US) through December 2021.[1] The Centers for Disease Control and Prevention recommends Coronavirus Disease 2019 (COVID-19) vaccination for all eligible persons, including those who have been previously infected.[2] As of December 2021, 71% of the US population has received at least one dose.[3] Vaccines approved in the US have a profile for mild to moderate adverse events, most of which are injection site pain, headache, fatigue, and myalgia.[4–6] Anecdotal reports emerged in 2021 suggesting that SARS-CoV-2 infection or COVID-19 vaccination could result in changes in menstrual cycles, raising widespread media attention and public interest, including a rapid response from the US National Institutes of Health by funding a COVID-19 and menstrual health network.[7–9] Concerns and unfounded information with regard to COVID-19 vaccination impairing reproductive health has also become a major reason for vaccine mistrust in the general population and vaccine hesitancy among children, adolescents, and women of reproductive age.[10–12] Therefore, the reproductive safety of COVID-19 vaccination is an emerging public health issue. Nevertheless, the impacts of SARS-CoV-2 infection and COVID-19 vaccination on menstrual health have not been systemically investigated.

Menstruation is tightly regulated by the hypothalamic-pituitary-ovary axis and can reflect women's overall health.[13] The menstrual cycle can be sensitive to a wide variety of inputs including stress, weight change, diet, and medication use, all of which changed during the COVID-19 pandemic.[14] Acute viral infection has been associated with disturbances in menstruation (e.g. dysmenorrhea, short or long cycles) through

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mechanisms involving immune dysregulation and direct inflammation of the ovaries.[15,16] New onset menstrual abnormalities have also been reported after vaccination against typhoid and hepatitis B.[17,18] Previous studies have found an increase in changes in menstrual cycle characteristics during the COVID-19 pandemic, or specifically after SARS-CoV-2 infection or COVID-19 vaccination, although findings are inconsistent.[19–28] However, this limited literature is severely hampered by cross-sectional designs that do not allow before and after comparisons, lack of comparison with uninfected or unvaccinated women, and fail to account for pandemic-related stress and behavioral changes.[19–28]

In this study, we prospectively examined the associations of SARS-CoV-2 infection and COVID-19 vaccination with changes in usual menstrual cycle characteristics, among pre-menopausal health professionals participating in an ongoing prospective cohort study. We additionally examined the duration of such changes, potential differences by vaccine type, and whether pandemic-related stress (e.g., distress, psychological wellbeing, local COVID-19 burden) accounted for changes in usual menstrual cycle characteristics.

Materials and Methods

Study design and population

The Nurses' Health Study 3 (NHS3) is an ongoing internet-based open cohort study established in 2010 which includes female and male nurses and nursing students from the US and Canada.[29] As of December 2021, 48,907 female and 856 male participants born on or after January 1, 1965 and aged ≥18 years had enrolled. Follow-up

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questionnaires are sent to participants approximately every 6 months. The most recent questionnaire (MOD12) was launched in July 2021 and contained questions regarding SARS-CoV-2 infection and COVID-19 vaccination status. MOD2 (baseline, starting 2011) and MOD12 (end of follow-up, December 2021) questionnaires collected information about current menstrual cycle characteristics. We also administered a series of supplementary questionnaires assessing health and well-being during the pandemic (COVID-19 sub-study) beginning in April 2020. The 12,323 participants (40% of 30,643 invited) who completed the first supplementary COVID-19 questionnaire were then asked to complete monthly and quarterly surveys through November 2021.

Women were eligible for this study if they completed the MOD2 and MOD12 questionnaires before December 8, 2021 (N=9,345), and had participated in the COVID-19 sub-study or completed COVID-19 questions in MOD12 (N=9,167). Women were excluded if they reported use of hormonal contraception in MOD12 (N=717), had reached menopause (N=2,836), had missing menstrual cycle information on either MOD2 (N=1,135) or MOD12 (N=13), were pregnant or had been pregnant in the 6 months preceding menstrual cycle assessment (N=607), or were unsure about vaccination status (blinded clinical trial participant, N=1), leaving 3,858 participants in the analysis (Figure 1). The study was approved by the Institutional Review Boards of Brigham and Women's Hospital and the Harvard School of Public Health. Return of the completed questionnaires implied consent.

Menstrual cycle characteristics

Pre-pandemic and pandemic usual menstrual cycle length and regularity were measured on the MOD2 and MOD12 questionnaires, respectively, using the same set

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of questions. Participants were asked to report the usual length (<21 days, 21-25 days, 26-31 days, 32-39 days, 40-50 days, more than 50 days or too irregular to count, no periods or amenorrhea) and regularity (very regular (within 3 days), regular (within 5-7 days), usually irregular, always irregular, no periods or amenorrhea) of their menstrual cycles. Change in menstrual cycle length and regularity was derived by calculating the difference in length or regularity that was reported at two time points: a pre-COVID assessment (MOD2) and a post-COVID assessment (MOD12). Self-report of usual menstrual cycle characteristics has been previously validated.[30,31]

SARS-CoV-2 infection and COVID-19 vaccination status

Date of a positive SARS-CoV-2 diagnostic test since March 1, 2020, was self-reported on MOD12 and each of the COVID-19 sub-study questionnaires. COVID-19 vaccination status, vaccine type, and date of the first dose were self-reported on MOD12 and the second and third quarterly follow-up of the COVID-19 sub-study.

Covariates

Demographic and socioeconomic characteristics including age, race/ethnicity, height, weight, education, smoking status, and country/state of residence were self-reported at enrollment. History of gynecological diseases was self-reported on MOD12. Current healthcare working status was reported on the COVID-19 sub-study baseline survey. Depressive symptoms, anxiety symptoms, perceived stress, post-traumatic stress disorder symptoms, and worry about COVID-19 was measured repeatedly in the COVID-19 sub-study (Table S1). County- and date-specific COVID-19 mortality data were used to derive a measure of time- and place-specific COVID-19 burden.[32]

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Statistical analysis

We first compared sociodemographic, behavioral, and mental health characteristics according to SARS-CoV-2 infection and COVID-19 vaccination status at the end of follow-up. To examine the cross-sectional associations between SARS-CoV-2 infection and COVID-19 vaccination and cycle characteristics in 2021, we fit logistic or multinomial logistic regression models with infection and vaccination each as the independent variable to estimate odds ratios (OR) and 95% confidence intervals (CIs) of categorized cycle characteristics in 2021, adjusting for demographic, behavioral, and socioeconomic factors, time of follow-up, pre-pandemic cycle characteristics, and mutually adjusting infection and vaccination status. Among 3,116 participants who were not using hormonal contraception at MOD2, we also examined changes in usual menstrual cycle length (no change, shorter, longer) and regularity (no change, more regular, less regular) during follow-up in relation to infection and vaccination status. In a sub-group of participants (N=2,209) with repeated measures of pandemic-related stress, we further adjusted for the most recently reported and highest level of stress during follow-up, respectively. Missing categorical values for covariates were assigned to a missing indicator, and median values were assigned for continuous variables of covariates with missing values (<1%). In secondary analyses, we stratified analyses by the timing between vaccination and report of usual menstrual cycle characteristics (0-6, 7-9, >9 months), and vaccination type (unvaccinated, mRNA (Pfizer/Moderna), Adenovirus-vectored (Janssen) to examine whether the change in usual menstrual cycle characteristics was transient and if there were potential differences by vaccine type. In this analysis, we excluded participants who reported receiving the AstraZeneca vaccine (n=9) and participants who did not report vaccine type (n=6). Last, we conducted four sensitivity analyses. First, we excluded 1354 women over 45 years at end of follow-up to minimize the effect of menstrual changes during the menopausal transition. Second, to reduce outcome misclassification, we excluded 119 women reporting 'no periods/amenorrhea' at any time point. Third, we excluded 348 women with self-reported gynecological conditions known to cause menstrual disorders including polycystic ovary syndrome, uterine fibroids, and endometriosis. Fourth, to examine whether women with irregular or short/long cycles were more susceptible to menstrual cycle changes, we stratified analyses by pre-pandemic menstrual cycle characteristics. All analyses were performed using SAS 9.4 (SAS Institute Inc). All statistical tests were two-sided, and statistical significance was defined as $P<0.05$.

Results

The 3,858 women were predominantly White (89.7%) and resided in the US (97.7%). Median age at baseline and end of follow-up was 33 years (range=21-51) and 42 years (range=27-56), respectively. Median follow-up time was 9.2 years. About 1 in 10 participants (N=421; 10.9%) reported a positive COVID-19 test between March 2020 and October 2021. Among these, 223 tested positive after first dose of vaccine. Most participants (N=3,527; 91.4%) were vaccinated by December 2021. Among women infected with SARS-CoV-2, the median time from infection to end of follow-up was 8.7 months (range=0.2-20.1). Among vaccinated participants, the median time from vaccination to end of follow-up was 8.5 months (range=1.0-13.4). Most women who were vaccinated during follow-up received an mRNA vaccine; women vaccinated with Adenovirus-vectored vaccines were vaccinated later in follow-up (Table S2). Compared

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to participants who never reported SARS-CoV-2 infection during follow-up, those who were infected were more likely to be Hispanic, reside in the Midwest and Southern US, have a higher BMI, be frontline healthcare workers, and less likely to be very worried about COVID-19 (Table 1). Compared to women who had not received the first dose of a COVID-19 vaccine, vaccinated participants were more likely to reside in the West and Northeastern US or outside of the US, have gained more weight during follow-up, have a higher educational attainment, be frontline healthcare workers, have higher levels of pandemic-related mental health distress, and reside in an area with greater COVID-19 mortality rate.

Before the pandemic, among non-oral contraceptive users, 18.6% women reported cycles ⩾32 days, and 15.0% reported irregular cycles. The corresponding figures were 17.6% and 22.7% in 2021. SARS-CoV-2 infection and vaccination status were not associated with cycle length or regularity in 2021 after adjusting for age, baseline cycle characteristics, and other factors (Table S3).

Overall, 2,227 women reported a change in either cycle length (N=1,408) or regularity (N=1,735) during follow-up. COVID-19 vaccination was associated with a 54% (OR=1.54, 95% CI: 1.14-2.27) higher risk of change to longer cycles in multivariable-adjusted models (Table S4, Table 2). SARS-CoV-2 infection was not associated with changes in cycle length or regularity. Results were similar after adjusting for cycle characteristics at baseline (Table S5), healthcare worker status, mental health status, and local COVID-19 burden (Table S6).

COVID-19 vaccination was associated with a change in cycle length only in the first 6 months after vaccination (0-6 months: OR=1.72, 95% CI=1.08-2.73; 7-9 months:

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OR=1.49, 95% CI=1.00-2.23; >9 months: OR=1.44, 95% CI=0.93-2.22, Table 3). The association of vaccination status with change in cycle length was similar for mRNA and Adenovirus-vectored vaccines (Table 4). There was a suggestion that Adenovirus-vectored vaccines were related to a higher likelihood of change in cycle regularity, but there were few women in this group (n=75), with a shorter duration of post-vaccination follow-up. We did not detect differences in the observed associations between brands of mRNA vaccines (Table S7).

Results were similar in analyses restricted to participants <45 years (Table S8), excluding participants reporting ‘no periods’ at any time points (Table S9), and excluding participants with gynecological conditions known to affect menstrual patterns (Table S10). Nevertheless, the association between COVID-19 vaccination and increased menstrual cycle length appeared to be driven by women reporting irregular or short/long (<26 or ⩾32 days) cycles at baseline (Table 5, Table S11-12).

Comment

Principal Findings

In this prospective study of 3,858 pre-menopausal women, COVID-19 vaccination was associated with an increase in usual menstrual cycle length, after adjusting for potential confounders including local COVID-19 burden and pandemic-related stressors. This change appeared to be limited to the 6 months following vaccination, and was strongest among women whose cycles were irregular, short, or long before the pandemic. This association was similar for Adenovirus-vectored and mRNA vaccines. SARS-CoV-2 infection was not associated with changes in usual menstrual characteristics.

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Results in the Context of What is Known

Menstrual health is known to change when challenged by psychosocial, interpersonal, or environmental stressors,[14,33] all of which occurred during the COVID-19 pandemic. Existing literature has generated conflicting results characterizing menstrual cycle profiles during the pandemic. Three previous studies observed a higher prevalence of menstrual cycle disruptions (e.g. irregularity, decreased duration of periods, more severe menstrual symptoms) during the pandemic compared with pre-pandemic data,[21,23,25] whereas another two studies found no change or a decreased incidence of menstrual disorders (e.g. anovulatory cycles, abnormal cycle lengths, prolonged menses).[19,26] However, these studies examined neither infection nor vaccination. SARS-CoV-2 infection has been associated with menstrual irregularity or abnormal bleeding in three retrospective studies.[24,34,35] In contrast, Ding et al. found no changes in menstrual characteristics, but a significant decline in ovarian reserve when comparing COVID-19 patients to age-matched uninfected women.[36] However, these previous studies were cross-sectional and provide limited information about changes from pre-pandemic cycle characteristics, or the temporality of change relative to infection or vaccination.

Our results suggest that COVID-19 vaccination results in a short-term increased risk of change in cycle length (longer cycles), independent of infection status. Menstrual disruptions have been reported shortly after typhoid and HBV vaccination, although mechanisms explaining these changes remains unexplored.[17,18] The United Kingdom (UK) Medicine and Healthcare products Regulatory Agency (MHRA) has documented over 40,000 suspected menstrual disorder cases following 50 million COVID-19 vaccine

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doses administered to women.[37] Three retrospective studies revealed that 20-50% of vaccinated women reported changes in cycle length or flow, with longer cycle length and heavier flows more commonly reported in all three studies.[20,27,38] To date, most of the reported menstrual cycle changes were short-lived.[27,37] Previous studies have yielded mixed results in investigating the associations between COVID-19 vaccine and menstrual cycle change. A retrospective study of 1,273 vaccinated women in the UK did not find associations between COVID-19 vaccination and changes in menstruation.[22] Another retrospective study of 5,000 premenopausal vaccinated women in the UK found that a history of positive COVID-19 test was associated with 49% (95% CI=20%-84%) increased risk of any cycle change following vaccination.[20] However, these studies lacked comparison with unvaccinated women, and did not exclude women who were using hormonal contraception. Similar to our findings, a prospective cohort study of 3,959 US women who had normal pre-vaccination menstrual cycle length (24-38 days) found that COVID-19 vaccination was associated with a small (<1 day) increase in usual cycle length compared with unvaccinated women, and such changes attenuated after two cycles.[28] Yet, this study did not account for SARS-CoV-2 infection or mental health or pandemic-related stressors. Moreover, none of these studies had measures of pre-pandemic menstrual cycle characteristics.

Clinical implications

We observed increases in usual cycle length after vaccination with both mRNA and adenovirus-vectored COVID-19 vaccines, suggesting shared mechanisms. There are multiple plausible mechanisms to explain the observed association, including immunological influences on sex hormones and systemic inflammatory responses that may invoke downstream responses in target organs.[39–41] A normal menstrual cycle is characterized by tightly regulated inflammatory and immune mediators, particularly matrix metalloproteinases, that facilitate the endometrial tissue breakdown and degradation needed for menstruation.[42–44] Furthermore, immune cell activation may contribute to heavy menstrual bleeding.[45] The immune response induced by both mRNA and adenovirus-vectored vaccines may temporarily affect the HPO axis, which could lead to menstrual disturbances.[46,47] More needs to be determined regarding the mechanisms by which inflammatory response to a vaccine effects the ovaries and uterus. We did not find evidence linking SARS-CoV-2 infection with subsequent menstrual changes, suggesting the short-term effect of vaccines might differ from the immune response to SARS-CoV-2 infection, which appears to be more extensive and tissue-specific than that elicited by vaccines.[48,49] However, due to the small number of infected participants in this study, this finding needs to be interpreted with caution.

Although not statistically significant, changes in cycle length after the Adenovirus-vectored vaccine appeared to be slightly stronger than those observed with mRNA vaccines. Given that the menstrual changes observed in our study were short-term, this apparent difference could be the result of earlier access to mRNA vaccines in this study population, rather than by a true difference in biological effects of different vaccine vectors. The difference in vaccination timing between mRNA and Adenovirus-vectored vaccines in our study, and the small number of women vaccinated with the latter (n=75), precluded us from evaluating whether the changes associated with the adenovirus-vectored vaccine were short-term as was the case in the aggregate data.

Strengths and Limitations

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Our study has limitations. Menstrual cycle characteristics and SARS-CoV-2 infection and vaccination were self-reported, although the validity of self-reported health information is high in cohorts of health professionals and menstrual characteristics have been demonstrated to be reported with strong accuracy.[50,51] That our study population was mainly healthcare workers with a high vaccination rate may limit generalizability to populations with a different pandemic experience including those with access to vaccinations later in the course of the pandemic. In addition, for many participants, our primary exposure and outcome were collected on the same questionnaire, so report of vaccination could have influenced report of menstrual cycle characteristics. However, this bias should be absent from the change in menstrual characteristic analyses that utilized pre-pandemic and post-exposure reports.

There are weaknesses in precision of our data. First, while access to menstrual cycle data that were reported prospectively prior to the pandemic is a strength for unbiased evaluation of change, these pre-pandemic cycle characteristics were reported 5-10 years prior to the pandemic and may not be representative of participants' menstrual cycles immediately prior to COVID-19 vaccination or infection. Second, we did not have information about the date of vaccination relative to ovulation. Because the uterine immune system changes with the menstrual cycle, date of inoculation relative to date of last menstrual period may be relevant.[22,40,44] Third, we only collected menstrual cycle length in categories, which improves validity but may have precluded us from detecting more subtle changes in cycle characteristics if they exist. Furthermore, we collected information about 'usual' cycle length. Thus, we may not have detected changes that were too mild or transient for participants to perceive and report them as newly "usual"

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cycle length or regularity. Any change not interpretated as "usual" would have biased our results towards an erroneous null association.

Our study has several strengths. Periodic surveys were administered over a 1-year period in the midst of the COVID-19 pandemic, rigorously measuring incident SARS-CoV-2 infection and COVID-19 vaccination, allowing comparison with those uninfected and unvaccinated. We were able to control for the impacts of pandemic on an individual's social functioning, mental health, and behavioral practices using validated measures. Menstrual cycle characteristics were collected prospectively throughout women's reproductive years prior to and during the COVID-19 pandemic, which allowed us to compare menstrual cycle characteristics before and after COVID-19 infection and vaccination.

Conclusions

We found that COVID-19 vaccination may be associated with a short-term change towards longer menstrual cycles. These changes were not explained by differences in health-related behavioral factors or pandemic-related stress. In addition, these menstrual disturbances did not appear to be related to vaccine type. Our findings suggest the need to monitor menstrual cycle health in vaccine clinical trials and increased attention to sex-based differences in vaccine response, especially in the setting of the rollout of COVID-19 boosters, which provides another opportunity to study this important issue. Future research will be needed to understand the underlying mechanisms for these associations.

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Author Contribution: SW analyzed and drafted the manuscript. SW, SAM and JEC were involved in the study conception and design. JM checked the accuracy of data analysis. All authors contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content and approved the final version of the manuscript. SW and JEC had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Data Availability: Further information including the procedures to obtain and access data from the Nurses' Health Studies is described at https://www.nurseshealthstudy.org/researchers (contact email: nhsaccess@channing.harvard.edu).

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Table 1 Demographic, socioeconomic, and health characteristics according to SARS-CoV-2 infection and COVID-19 vaccination status, between 2011 and Dec 8, 2021, the Nurses' Health Study 3 (NHS3), N=3,858

	SARS-CoV-2 infection		COVID-19 vaccination	
	No (n=3437)	Yes (n=421)	No (n=331)	Yes (n=3527)
Age at baseline[a], mean (SD), years	33.4 (6.3)	33.4 (6.6)	32.5 (5.9)	33.4 (6.3)
Age at end of follow up[b], mean (SD), years	42.4 (6.3)	42.4 (6.7)	41.8 (5.9)	42.5 (6.4)
Race/Ethnicity, n (%)				
Hispanic	133 (3.9)	19 (4.5)	18 (5.4)	134 (3.8)
Non-Hispanic White	3082 (89.7)	378 (89.8)	283 (85.5)	3177 (90.1)
Others	205 (6.0)	18 (4.3)	27 (8.2)	196 (5.6)
Region, n (%)				
West	813 (23.7)	69 (16.4)	67 (20.2)	815 (23.1)
Midwest	941 (27.4)	146 (34.7)	120 (36.3)	967 (27.4)
South	714 (20.8)	107 (25.4)	83 (25.1)	738 (20.9)
Northeast	818 (23.8)	91 (21.6)	52 (15.7)	857 (24.3)
Military or outside of US	151 (4.4)	8 (1.9)	9 (2.7)	150 (4.3)
BMI at baseline[a], mean (SD), kg/m^2	25.5 (5.9)	27.2 (7.0)	25.8 (5.8)	25.7 (6.1)
BMI at end of follow up[b], mean (SD), kg/m^2	27.4 (8.8)	29.2 (7.7)	27.4 (6.2)	27.6 (8.9)
Weight change, mean (SD), pounds	11.1 (39.0)	12.0 (24.7)	9.6 (20.2)	11.3 (39.0)
Smoking status, n (%)				
Never	3262 (95.0)	396 (94.1)	307 (92.8)	3351 (95.1)
Past	87 (2.5)	16 (3.8)	12 (3.6)	91 (2.6)
Current	85 (2.5)	9 (2.1)	12 (3.6)	82 (2.3)
Education attainment, n (%)				
Nursing student/Diploma in Nursing/Associate's Degree	198 (5.8)	27 (6.4)	49 (14.8)	176 (5.0)
Bachelor's Degree	1372 (39.9)	171 (40.6)	160 (48.3)	1383 (39.2)
Master's Degree	1478 (43.0)	183 (43.5)	97 (29.3)	1564 (44.4)
Doctorate Degree	389 (11.3)	40 (9.5)	25 (7.6)	404 (11.4)
COVID-19 sub-study participants only	No (n=2653)	Yes (n=338)	No (n=249)	Yes (n=2742)
Frontline healthcare worker, n (%)[c]	2001 (75.4)	279 (82.5)	176 (70.6)	2104 (76.7)

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Depressive symptoms (PHQ-2), mean (SD)[d]	2.4 (1.7)	2.5 (1.7)	2.0 (1.6)	2.5 (1.7)
Anxiety symptoms (GAD-2), mean (SD)[d]	3.1 (1.7)	3.1 (1.6)	2.6 (1.7)	3.2 (1.7)
Posttraumatic stress symptoms (IES-6), mean (SD)[d]	1.8 (0.8)	1.7 (0.8)	1.4 (0.8)	1.8 (0.8)
Perceived stress (PSS-4), mean (SD)[d]	6.7 (3.0)	6.6 (3.0)	6.1 (2.9)	6.8 (3.0)
Worry about COVID-19, n (%)[d]				
Not at all	25 (1.0)	3 (0.9)	14 (5.6)	14 (0.5)
Not very worried	225 (8.5)	39 (11.6)	79 (31.7)	185 (6.8)
Somewhat worried	1492 (56.5)	199 (59.1)	129 (51.8)	1562 (57.2)
Very worried	901 (34.1)	96 (28.5)	27 (10.8)	970 (35.5)
Residential county COVID-19 mortality/10,000, n (%)[d,e]				
0.00	395 (15.7)	59 (18.0)	54 (22.5)	400 (15.4)
>0.00–<0.25	763 (30.3)	83 (25.3)	53 (22.1)	793 (30.4)
0.25–<0.75	621 (24.7)	91 (27.7)	74 (30.8)	638 (24.5)
0.75-7.37	739 (29.4)	95 (29.0)	59 (24.6)	775 (29.7)

Values of polytomous variables may not sum to 100% due to missingness. Missingness was 0.7% for BMI, 0.6% for race/ethnicity. PHQ-2, 2-item Patient Health Questionnaire. GAD-2, 2-item Generalized Anxiety Disorder. IES-6, 6-item Impact of Events Scale, adapted to be specific to COVID-19 trauma. PSS-4, 4-item Perceived Stress Scale

[a] MOD-2 (2011-2016)

[b] MOD-12 (2021)

[c] Physically working at a site providing clinical care

[d] Highest psychological distress level during follow-up.

[e] County- and date-specific COVID-19 mortality data on the date of questionnaire return from the COVID-19 Data Repository by the Center for Systems Science and Engineering at Johns Hopkins University were used to derive a measure of local COVID-19 burden

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Table 2. Odds Ratios (OR) and 95% confidence intervals (CI) of change in menstrual cycle characteristics in relation to SARS-CoV-2 infection and COVID-19 vaccination status, the Nurses' Health Study 3 (NHS3), N=3,116

	SARS-CoV-2 infection[a]	COVID-19 vaccination[b]
	N=349	N=2835
Change in usual menstrual characteristics	OR (95% CI)	OR (95% CI)
Change in cycle length or regularity		
Any change (n=2227)	0.84 (0.66-1.08)	1.10 (0.84-1.45)
Change in cycle length		
Any change (n=1408)	0.89 (0.71-1.12)	1.27 (0.98-1.65)
Change in cycle length[c]		
Shorter (n=858)	0.89 (0.68-1.16)	1.17 (0.88-1.57)
Longer (n=550)	0.89 (0.64-1.22)	1.48 (1.00-2.19)
Change in cycle regularity		
Any change (n=1735)	0.90 (0.72-1.14)	0.86 (0.67-1.12)
Change in cycle regularity[c]		
More regular (n=709)	0.81 (0.60-1.10)	0.89 (0.65-1.22)
Less regular (n=1026)	0.97 (0.74-1.27)	0.83 (0.61-1.12)

[a] Reference: uninfected
[b] Reference: unvaccinated
[c] Multinomial logistic model
Change in cycle characteristics defined by change in three categories: cycle regularity: i) very regular [+-3 days], ii) regular [within 5-7 days]; iii) usually irregular or always irregular or no periods; cycle length: i) <26 days; ii) 26-31 days; iii) ⩾32 days.
Models adjusted for age at baseline, follow up time, BMI at end of follow up, weight change, race/ethnicity, education attainment, region, and mutually adjusting for SARS-CoV-2 infection and vaccination
742 hormonal contraception users at MOD2 were excluded from analysis

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Table 3 Odds Ratios (OR) and 95% confidence intervals (CI) of change in menstrual cycle characteristics in relation to SARS-CoV-2 infection and COVID-19 vaccination status, stratifying by the timing between infection or vaccination and end-of follow-up assessment of cycle characteristics, the Nurses' Health Study 3 (NHS3), N=3,116

Change in menstrual characteristics	SARS-CoV-2 infection				COVID-19 vaccination			
	Uninfected	0-6 months from infection	7-9 months from infection	>9 months from infection	Unvaccinated	0-6 months from vaccination	7-9 months from vaccination	>9 months from vaccination
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
	N=2767	N=56	N=130	N=159	N=281	N=392	N=1541	N=641
Change in cycle length or regularity								
Any change	Ref [1.0]	0.52 (0.30-0.90)	1.06 (0.71-1.60)	0.88 (0.62-1.24)	Ref [1.0]	1.32 (0.93-1.87)	1.06 (0.80-1.42)	1.01 (0.74-1.39)
Change in cycle length								
Any change	Ref [1.0]	0.75 (0.43-1.31)	0.93 (0.65-1.34)	0.92 (0.66-1.27)	Ref [1.0]	1.40 (1.02-1.92)	1.30 (0.99-1.69)	1.20 (0.89-1.61)
Change in cycle length[a]								
Shorter	Ref [1.0]	0.56 (0.28-1.15)	0.91 (0.59-1.40)	1.03 (0.71-1.48)	Ref [1.0]	1.26 (0.87-1.80)	1.23 (0.91-1.66)	1.10 (0.79-1.54)
Longer	Ref [1.0]	1.13 (0.56-2.29)	0.94 (0.58-1.54)	0.72 (0.44-1.18)	Ref [1.0]	1.67 (1.05-2.64)	1.43 (0.96-2.14)	1.41 (0.91-2.18)
Change in cycle regularity								
Any change	Ref [1.0]	0.70 (0.41-1.21)	0.97 (0.67-1.39)	0.94 (0.68-1.31)	Ref [1.0]	0.88 (0.64-1.20)	0.84 (0.65-1.10)	0.85 (0.64-1.14)
Change in cycle regularity[a]								
More regular	Ref [1.0]	0.61 (0.29-1.26)	0.97 (0.60-1.54)	0.81 (0.52-1.24)	Ref [1.0]	0.89 (0.60-1.33)	0.89 (0.64-1.24)	0.81 (0.56-1.16)
Less regular	Ref [1.0]	0.81 (0.43-1.52)	0.96 (0.63-1.47)	1.04 (0.71-1.53)	Ref [1.0]	0.83 (0.57-1.21)	0.78 (0.57-1.07)	0.88 (0.62-1.24)

[a] Multinomial logistic model

Change in cycle characteristics defined by change in three categories: cycle regularity: i) very regular [+-3 days], ii) regular [within 5-7 days]; iii) usually irregular or always irregular or no periods; cycle length: i) <26 days; ii) 26-31 days; iii) ⩾32 days.

Models adjusted for age at baseline, follow up time, BMI at end of follow up, weight change, race/ethnicity, education attainment, region, and mutually adjusting for SARS-CoV-2 infection and vaccination

742 hormonal contraception users at MOD2 were excluded from analysis; 4 participants missing date of infection; 261 participants missing date of vaccination

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Table 4 Odds Ratios (OR) and 95% confidence intervals (CI) of change in menstrual cycle characteristics in relation to COVID-19 vaccination status according to vaccine type, the Nurses' Health Study 3 (NHS3), N=3,102

	COVID-19 vaccination		
	Unvaccinated	mRNA vaccine[a]	Adenovirus-vectored vaccine[b]
Change in menstrual characteristics	OR (95% CI)	OR (95% CI)	OR (95% CI)
	N=281	N=2746	N=75
Change in cycle length or regularity			
Any change	Ref [1.0]	1.08 (0.82-1.42)	2.00 (1.04-3.85)
Change in cycle length			
Any change	Ref [1.0]	1.26 (0.97-1.63)	1.42 (0.85-2.39)
Change in cycle length[c]			
Shorter	Ref [1.0]	1.16 (0.86-1.55)	1.25 (0.69-2.28)
Longer	Ref [1.0]	1.47 (1.00-2.17)	1.78 (0.88-3.60)
Change in cycle regularity			
Any change	Ref [1.0]	0.84 (0.65-1.09)	1.97 (1.11-3.51)
Change in cycle regularity[c]			
More regular	Ref [1.0]	0.86 (0.63-1.18)	2.20 (1.12-4.33)
Less regular	Ref [1.0]	0.81 (0.60-1.10)	1.80 (0.94-3.44)

[a] Pfizer, Moderna

[b] Johnson & Johnson (Jansen)

[c] Multinomial logistic model

Change in cycle characteristics defined by change in three categories: cycle regularity: i) very regular [+-3 days], ii) regular [within 5-7 days]; iii) usually irregular or always irregular or no periods; cycle length: i) <26 days; ii) 26-31 days; iii) ⩾32 days.

Models adjusted for age at baseline, follow up time, BMI at end of follow up, weight change, race/ethnicity, education attainment, region, and SARS-CoV-2 infection

742 hormonal contraception users at MOD2, 9 participants who reported AstraZeneca vaccine, and 6 with unknown vaccine type were excluded from analysis

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Table 5. Odds Ratios (OR) and 95% confidence intervals (CI) of change in menstrual cycle characteristics in relation to SARS-CoV-2 infection and COVID-19 vaccination status, stratified by menstrual cycle characteristics at baseline (participants who had regular (within 7 days) and normal cycle length (26-31 days), (N=1976) versus irregular (>7 days) or short/long cycle length (<26 or ⩾32 days), (N=1,140)), the Nurses' Health Study 3 (NHS3)

	SARS-CoV-2 infection[a]		COVID-19 vaccination[b]	
	Baseline menstrual cycle characteristics		Baseline menstrual cycle characteristics	
	Regular and normal length	Irregular or short or long cycle	Regular and normal length	Irregular or short or long cycle
Change in usual menstrual characteristics	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Change in cycle length or regularity				
Any change	0.82 (0.61-1.11)	0.90 (0.56-1.46)	0.94 (0.66-1.32)	1.83 (1.12-3.00)
Change in cycle length				
Any change	0.80 (0.58-1.10)	1.02 (0.70-1.50)	1.19 (0.83-1.71)	1.69 (1.12-2.54)
Change in cycle length[c]				
Shorter	0.85 (0.58-1.22)	0.97 (0.62-1.50)	1.33 (0.87-2.05)	1.32 (0.85-2.06)
Longer	0.68 (0.42-1.11)	1.10 (0.68-1.77)	0.95 (0.56-1.60)	2.82 (1.51-5.27)
Change in cycle regularity				
Any change	0.89 (0.66-1.20)	0.97 (0.66-1.42)	0.77 (0.55-1.08)	1.05 (0.69-1.59)
Change in cycle regularity[c]				
More regular	0.82 (0.52-1.30)	0.83 (0.54-1.29)	0.82 (0.51-1.34)	1.05 (0.66-1.66)
Less regular	0.90 (0.64-1.25)	1.22 (0.76-1.97)	0.74 (0.51-1.08)	1.01 (0.59-1.74)

[a] Reference: uninfected
[b] Reference: unvaccinated
[c] Multinomial logistic model
Change in cycle characteristics defined by change in three categories: cycle regularity: i) very regular [+-3 days], ii) regular [within 5-7 days]; iii) usually irregular or always irregular or no periods; cycle length: i) <26 days; ii) 26-31 days; iii) ⩾32 days.
Multivariable model adjusting for age at baseline, and follow up time, BMI at end of follow up, weight change, race/ethnicity, education attainment, region, and mutually adjusting for SARS-CoV-2 infection and COVID-19 vaccination with each other
742 hormonal contraception users at MOD2 were excluded from analysis

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Figure 1 Flowchart of the study population selection, the Nurses' Health Study 3 (NHS3)

Note. Participants were asked to report the usual length (<21 days, 21-25 days, 26-31 days, 32-39 days, 40-50 days, more than 50 days or too irregular to count, no periods or amenorrhea) and regularity (very regular (within 3 days), regular (within 5-7 days), usually irregular, always irregular, no periods or amenorrhea) of their menstrual cycle.

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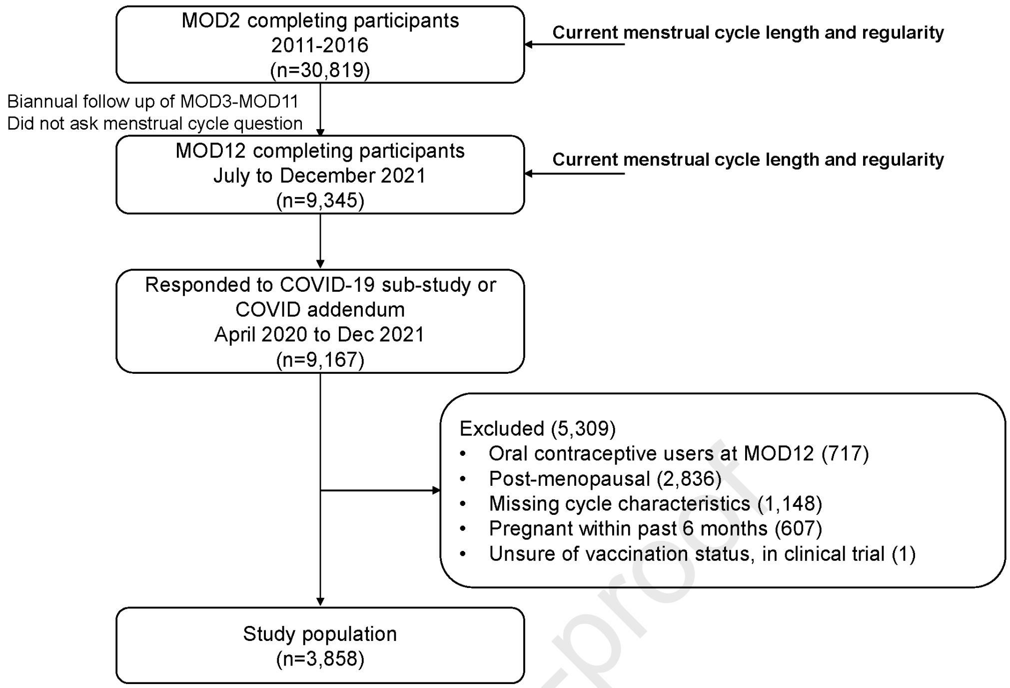

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Table S1 Measure of mental health during the pandemic in the COVID-19 sub-study surveys

Construct	Measure	Questionnaire Time Point	Derivation
Depressive Symptoms	2-item Patient Health Questionnaire	COVID-19 sub-study baseline, Monthly 1, 2, 3, Quarterly 1, 2, 3	Sum scores were derived, with higher sore indicating more severe symptoms
Anxiety Symptoms	2-item Generalized Anxiety Disorder	COVID-19 sub-study baseline, Monthly 1, 2, 3, Quarterly 1, 2, 3	Sum scores were derived, with higher sore indicating more severe symptoms
Posttraumatic Stress Symptoms	6-item Impact of Events Scale, adapted to be specific to COVID-19 trauma	COVID-19 sub-study baseline, Monthly 1, 2, 3, Quarterly 1, 2, 3	Mean scores derived, with higher sore indicating more severe symptoms
Worry about COVID	Single question derived from a national poll at YouGov[a]	COVID-19 sub-study baseline, Monthly 1, 2, 3, Quarterly 1, 3	Not at all worried and not very worried versus somewhat worried and very worried
Perceived stress	4-item Perceived Stress Scale	COVID-19 sub-study baseline, Monthly 1, 2, 3, Quarterly 1	Sum scores were derived, with higher sore indicating higher stress

[a] https://docs.cdn.yougov.com/1ayt0i64g6/20200408_yahoo_coronavirus.pdf

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Table S2. Vaccine brand and time from vaccination to end of follow-up among vaccinated participants, the Nurses' Health Study 3 N=3,526

	N (%)	Time between vaccination[a] to end of follow up, median (range), days
Pfizer	2145 (60.8)	258 (31-403)
Moderna	1282 (36.4)	230 (44-401)
Jansen (Johnson & Johnson)	84 (2.4)	159 (33-386)
AstraZeneca/Oxford	9 (0.3)	204 (139-260)
Unknown	7 (0.2)	208 (106-279)

[a] Vaccination date was defined as the date of the first dose of a two-dose vaccine or the date of vaccination for single-dose vaccines.

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Table S3. Odds Ratios (OR) and 95% confidence intervals (CI) of categorized usual menstrual cycle characteristics at the end of follow-up (July-Dec 2021) in relation to SARS-CoV-2 infection and COVID-19 vaccination, the Nurses' Health Study 3 (NHS3), N=3,858

Usual menstrual characteristic	SARS-CoV-2 infection[a] N=421			COVID-19 vaccination[b] N=3527		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Cycle length[c]						
<26 days (n=848)	1.05 (0.81-1.36)	1.05 (0.81-1.36)	1.05 (0.81-1.36)	0.91 (0.68-1.20)	0.96 (0.72-1.29)	0.97 (0.73-1.29)
⩾32 days (n=680)	1.16 (0.88-1.53)	1.13 (0.85-1.49)	1.13 (0.85-1.49)	0.99 (0.72-1.37)	1.00 (0.72-1.39)	1.01 (0.73-1.40)
Cycle regularity[d]						
Irregular (n=876)	1.17 (0.90-1.51)	1.14 (0.88-1.48)	1.15 (0.89-1.49)	1.14 (0.84-1.55)	1.18 (0.86-1.61)	1.19 (0.87-1.63)

[a] Reference: uninfected
[b] Reference: unvaccinated
Model 1: adjusting for age at baseline, and follow up time
Model 2: model 1 + adjusting for BMI at end of follow up, weight change, race/ethnicity, education attainment, and region
Model 3: model 2 + mutually adjusting for SARS-CoV-2 infection and vaccination
[c] multinomial logistic model, additionally adjusted cycle length at baseline (<26 days, 26-31 days, ⩾32 days, hormonal contraception users)
[d] additionally adjusted for cycle regularity at baseline (regular (very regular or regular), irregular (usually irregular, always irregular, or no periods), hormonal contraception users)

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Table S4. Frequency of change in usual menstrual cycle length and regularity in relation to SARS-CoV-2 infection and COVID-19 vaccination, the Nurses' Health Study 3 (NHS3), N=3,116

	SARS-CoV-2 infection		COVID-19 vaccination	
	No (n=2767)	Yes (n=349)	No (n=281)	Yes (n=2835)
Change in usual menstrual characteristics				
Change in cycle length[c]				
Shorter (n=858)	769 (27.8)	89 (25.5)	76 (27.1)	782 (27.6)
Longer (n=550)	492 (17.8)	58 (16.6)	35 (12.5)	515 (18.2)
Change in cycle regularity				
More regular (n=709)	639 (23.1)	70 (20.1)	71 (25.3)	638 (22.5)
Less regular (n=1026)	909 (32.9)	117 (33.5)	92 (32.7)	934 (33.0)

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Table S5. Odds Ratios (OR) and 95% confidence intervals (CI) of change in menstrual cycle characteristics in relation to SARS-CoV-2 infection and COVID-19 vaccination status, the Nurses' Health Study 3 (NHS3), additionally adjusting for menstrual cycle characteristics at baseline (participants who had regular (within 7 days) and normal cycle length (26-31 days), (N=1976) versus irregular (>7 days) or short/long cycle length (<26 or ⩾32 days), N=3,116

	SARS-CoV-2 infection[a]	COVID-19 vaccination[b]
	N=349	N=2835
Change in usual menstrual characteristics	OR (95% CI)	OR (95% CI)
Change in cycle length or regularity		
Any change (n=2227)	0.82 (0.64-1.06)	1.15 (0.86-1.52)
Change in cycle length		
Any change (n=1408)	0.87 (0.68-1.10)	1.35 (1.03-1.77)
Change in cycle length[c]		
Shorter (n=858)	0.87 (0.66-1.15)	1.25 (0.93-1.69)
Longer (n=550)	0.86 (0.62-1.19)	1.57 (1.05-2.33)
Change in cycle regularity		
Any change (n=1735)	0.90 (0.71-1.13)	0.87 (0.68-1.13)
Change in cycle regularity[c]		
More regular (n=709)	0.79 (0.58-1.08)	0.93 (0.67-1.29)
Less regular (n=1026)	0.98 (0.75-1.28)	0.82 (0.60-1.11)

[a] Reference: uninfected
[b] Reference: unvaccinated
[c] Multinomial logistic model
Change in cycle characteristics defined by change in three categories: cycle regularity: i) very regular [+-3 days], ii) regular [within 5-7 days]; iii) usually irregular or always irregular or no periods; cycle length: i) <26 days; ii) 26-31 days; iii) ⩾32 days.
Models adjusted for age at baseline, follow up time, BMI at end of follow up, weight change, race/ethnicity, education attainment, region, baseline menstrual cycle characteristics, and mutually adjusting for SARS-CoV-2 infection and vaccination
742 hormonal contraception users at MOD2 were excluded from analysis

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Table S6. Odds Ratios (ORs) and 95% confidence intervals (CI) of change in menstrual cycle characteristics in relation to SARS-CoV-2 infection and COVID-19 vaccination status, among COVID-19 sub-study respondents with repeated measure of mental health conditions during follow up, N=2,209

	SARS-CoV-2 infection[a]			COVID-19 vaccination[b]		
	N=265			N=2016		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
Change in menstrual characteristics	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Change in cycle length or regularity						
Any change (n=1582)	0.95 (0.71-1.28)	0.96 (0.72-1.29)	0.97 (0.72-1.29)	1.11 (0.79-1.54)	1.14 (0.81-1.62)	1.08 (0.76-1.52)
Change in cycle length						
Any change (n=997)	0.94 (0.72-1.23)	0.94 (0.72-1.23)	0.95 (0.73-1.24)	1.28 (0.94-1.74)	1.37 (0.99-1.90)	1.29 (0.93-1.78)
Change in cycle length[c]						
Shorter (n=608)	0.95 (0.70-1.29)	0.96 (0.70-1.31)	0.97 (0.71-1.32)	1.25 (0.87-1.80)	1.30 (0.90-1.90)	1.21 (0.83-1.77)
Longer (n=389)	0.90 (0.63-1.31)	0.89 (0.62-1.30)	0.91 (0.62-1.31)	1.29 (0.83-2.00)	1.48 (0.93-2.35)	1.38 (0.87-2.18)
Change in cycle length[d]						
Longer (n=389)	0.89 (0.61-1.29)	0.86 (0.59-1.25)	0.88 (0.61-1.28)	1.34 (0.86-2.08)	1.58 (0.99-2.52)	1.48 (0.93-2.35)
Change in cycle regularity						
Any change (n=1242)	0.93 (0.71-1.21)	0.93 (0.71-1.22)	0.93 (0.71-1.21)	0.79 (0.58-1.08)	0.75 (0.54-1.04)	0.74 (0.53-1.02)
Change in cycle regularity[c]						
More regular (n=499)	0.78 (0.55-1.11)	0.79 (0.56-1.13)	0.79 (0.55-1.13)	0.88 (0.59-1.30)	0.84 (0.56-1.26)	0.78 (0.52-1.17)
Less regular (n=743)	1.02 (0.75-1.39)	1.02 (0.75-1.40)	1.01 (0.74-1.38)	0.71 (0.50-1.02)	0.67 (0.46-0.97)	0.68 (0.47-1.00)

[a] Reference: uninfected
[b] Reference: unvaccinated
[c] Multinomial logistic model
[d] Excluding from analysis women who reported a change towards shorter length
Change in cycle characteristics defined by change in three categories: cycle regularity: i) very regular [+-3 days], ii) regular [within 5-7 days]; iii) usually irregular or always irregular or no periods; cycle length: i) <26 days; ii) 26-31 days; iii) ⩾32 days.
Model 1: adjusting for age at baseline, follow up time, BMI at end of follow up, weight change, race/ethnicity, education attainment, region, and mutually adjusting for SARS-CoV-2 infection and vaccination
Model 2: Model 1 + healthcare worker status, highest level of depressive symptoms (PHQ-2), anxiety symptoms (GAD-2), PTSD symptoms (IES-6), perceived stress (PSS-4), worry about COVID, county-level mortality during follow up
Model 3: Model 1 + healthcare worker status, most recent depressive symptoms (PHQ-2), anxiety symptoms (GAD-2), PTSD symptoms (IES-6), perceived stress (PSS-4), worry about COVID, county-level mortality
742 hormonal contraception users at MOD2 were excluded from analysis

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Table S7. Odds Ratios (ORs) and 95% confidence intervals (CI) of change in menstrual cycle characteristics in relation to SARS-CoV-2 infection and COVID-19 vaccination status, stratifying by vaccine brand, N=3,102

	COVID-19 vaccination			
	Unvaccinated	Pfizer	Moderna	Adenovirus-vectored vaccine[a]
Change in menstrual characteristics	OR (95% CI) N=281	OR (95% CI) N=1709	OR (95% CI) N=1037	OR (95% CI) N=75
Change in cycle length or regularity				
Any change	Ref [1.0]	1.08 (0.81-1.43)	1.08 (0.81-1.46)	2.00 (1.04-3.85)
Change in cycle length				
Any change	Ref [1.0]	1.28 (0.99-1.67)	1.24 (0.94-1.63)	1.43 (0.85-2.39)
Change in cycle length[b]				
Shorter	Ref [1.0]	1.20 (0.89-1.62)	1.11 (0.81-1.52)	1.25 (0.69-2.29)
Longer	Ref [1.0]	1.46 (0.98-2.18)	1.49 (0.99-2.25)	1.78 (0.88-3.60)
Change in cycle length[c]				
Longer	Ref [1.0]	1.53 (1.03-2.29)	1.53 (1.01-2.31)	1.82 (0.90-3.70)
Change in cycle regularity				
Any change	Ref [1.0]	0.86 (0.66-1.11)	0.83 (0.63-1.09)	1.97 (1.11-3.51)
Change in cycle regularity[b]				
More regular	Ref [1.0]	0.91 (0.65-1.26)	0.80 (0.56-1.13)	2.21 (1.12-4.34)
Less regular	Ref [1.0]	0.80 (0.59-1.10)	0.83 (0.60-1.15)	1.80 (0.94-3.45)

[a] Adenovirus-vector vaccine: Johnson & Johnson (Jansen)

[b] Multinomial logistic model

[c] Excluding from analysis women who reported a change towards shorter length

Change in cycle characteristics defined by change in three categories: cycle regularity: i) very regular [+-3 days], ii) regular [within 5-7 days]; iii) usually irregular or always irregular or no periods; cycle length: i) <26 days; ii) 26-31 days; iii) ⩾32 days.

Models adjusted for age at baseline, follow up time, BMI at end of follow up, weight change, race/ethnicity, education attainment, region, and SARS-CoV-2 infection

742 hormonal contraception users at MOD2, 9 participants who reported AstraZeneca vaccine, and 7 with unknown vaccine type were excluded from analysis

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Table S8. Odds Ratios (ORs) and 95% confidence intervals (CI) of change in menstrual cycle characteristics in relation to SARS-CoV-2 infection and COVID-19 vaccination status, excluding participants aged 45 years old or older at end of follow up, the Nurses' Health Study 3, N=1,762

	SARS-CoV-2 infection[a]			COVID-19 vaccination[b]		
	N=199			N=1580		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
Change in menstrual characteristics	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Change in cycle length or regularity						
Any change (n=1167)	0.93 (0.68-1.27)	0.93 (0.68-1.27)	0.93 (0.68-1.27)	1.03 (0.74-1.43)	1.03 (0.74-1.43)	1.02 (0.74-1.43)
Change in cycle length						
Any change (n=735)	1.00 (0.74-1.35)	1.03 (0.76-1.39)	1.04 (0.76-1.41)	1.16 (0.85-1.60)	1.18 (0.85-1.63)	1.18 (0.85-1.63)
Change in cycle length[c]						
Shorter (n=526)	0.94 (0.67-1.32)	0.97 (0.69-1.37)	0.97 (0.69-1.37)	1.03 (0.73-1.45)	1.06 (0.75-1.50)	1.06 (0.74-1.50)
Longer (n=209)	1.15 (0.74-1.81)	1.18 (0.75-1.86)	1.21 (0.77-1.92)	1.69 (0.95-3.02)	1.65 (0.92-2.96)	1.67 (0.93-3.01)
Change in cycle length[d]						
Longer (n=209)	1.15 (0.73-1.80)	1.17 (0.74-1.84)	1.19 (0.75-1.88)	1.67 (0.94-2.99)	1.63 (0.90-2.92)	1.64 (0.91-2.95)
Change in cycle regularity						
Any change (n=873)	0.98 (0.73-1.32)	0.97 (0.72-1.32)	0.96 (0.71-1.30)	0.82 (0.60-1.12)	0.82 (0.60-1.12)	0.82 (0.60-1.12)
Change in cycle regularity[c]						
More regular (n=510)	0.86 (0.60-1.23)	0.86 (0.60-1.24)	0.86 (0.60-1.23)	0.89 (0.62-1.28)	0.90 (0.62-1.30)	0.89 (0.61-1.29)
Less regular (n=363)	1.17 (0.81-1.69)	1.15 (0.79-1.68)	1.13 (0.77-1.65)	0.74 (0.51-1.10)	0.73 (0.49-1.08)	0.74 (0.49-1.09)

[a] Reference: uninfected
[b] Reference: unvaccinated
[c] Multinomial logistic model
[d] Excluding from analysis women who reported a change towards shorter length
Change in cycle characteristics defined by change in three categories: cycle regularity: i) very regular [+-3 days], ii) regular [within 5-7 days]; iii) usually irregular or always irregular; cycle length: i) <26 days; ii) 26-31 days; iii) ⩾32 days.
Model 1: adjusting for age at baseline, and follow up time
Model 2: model 1 + adjusting for BMI at end of follow up, weight change, race/ethnicity, education attainment, and region
Model 3: model 2 + mutually adjusting for SARS-CoV-2 infection and vaccination
742 hormonal contraception users at MOD2 were excluded from analysis

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Table S9. Odds Ratios (ORs) and 95% confidence intervals (CI) of change in menstrual cycle characteristics in relation to SARS-CoV-2 infection and COVID-19 vaccination status, excluding participants reported 'no periods/amenorrhea' at any time point, the Nurses' Health Study 3, N=2,997

	SARS-CoV-2 infection[a]			COVID-19 vaccination[b]		
	N=336			N=2721		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
Change in menstrual characteristics	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Change in cycle length or regularity						
Any change (n=2115)	0.83 (0.65-1.06)	0.83 (0.65-1.07)	0.84 (0.65-1.07)	1.09 (0.83-1.42)	1.10 (0.84-1.44)	1.08 (0.82-1.43)
Change in cycle length						
Any change (n=1335)	0.86 (0.69-1.09)	0.88 (0.69-1.11)	0.89 (0.70-1.12)	1.29 (1.00-1.66)	1.31 (1.01-1.70)	1.30 (1.00-1.69)
Change in cycle length[c]						
Shorter (n=511)	0.85 (0.65-1.11)	0.87 (0.66-1.14)	0.88 (0.67-1.15)	1.16 (0.87-1.54)	1.19 (0.89-1.60)	1.18 (0.88-1.59)
Longer (n=824)	0.88 (0.64-1.21)	0.88 (0.64-1.22)	0.90 (0.65-1.25)	1.59 (1.07-2.36)	1.58 (1.06-2.37)	1.57 (1.05-2.35)
Change in cycle length[d]						
Longer (n=824)	0.88 (0.64-1.22)	0.88 (0.63-1.22)	0.90 (0.65-1.25)	1.63 (1.10-2.43)	1.64 (1.09-2.45)	1.62 (1.08-2.43)
Change in cycle regularity						
Any change (n=1632)	0.94 (0.75-1.19)	0.94 (0.75-1.19)	0.93 (0.74-1.17)	0.85 (0.66-1.09)	0.85 (0.66-1.10)	0.85 (0.65-1.09)
Change in cycle regularity[c]						
More regular (n=688)	0.83 (0.61-1.12)	0.82 (0.61-1.12)	0.82 (0.60-1.11)	0.87 (0.64-1.19)	0.89 (0.65-1.22)	0.88 (0.64-1.21)
Less regular (n=944)	1.02 (0.78-1.34)	1.03 (0.79-1.35)	1.02 (0.78-1.33)	0.82 (0.61-1.10)	0.81 (0.60-1.09)	0.81 (0.60-1.09)

[a] Reference: uninfected
[b] Reference: unvaccinated
[c] Multinomial logistic model
[d] Excluding from analysis women who reported a change towards shorter length
Change in cycle characteristics defined by change in three categories: cycle regularity: i) very regular [+-3 days], ii) regular [within 5-7 days]; iii) usually irregular or always irregular; cycle length: i) <26 days; ii) 26-31 days; iii) ⩾32 days.
Model 1: adjusting for age at baseline, and follow up time
Model 2: model 1 + adjusting for BMI at end of follow up, weight change, race/ethnicity, education attainment, and region
Model 3: model 2 + mutually adjusting for SARS-CoV-2 infection and vaccination
742 hormonal contraception users at MOD2 were excluded from analysis

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Table S10. Odds Ratios (ORs) and 95% confidence intervals (CI) of change in menstrual cycle characteristics in relation to SARS-CoV-2 infection and COVID-19 vaccination status, excluding participants with self-reported polycystic ovary syndrome, uterine fibroids, or endometriosis, the Nurses' Health Study 3, N=2,768

	SARS-CoV-2 infection[a]			COVID-19 vaccination[b]		
	N=308			N=2521		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
Change in menstrual characteristics	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Change in cycle length or regularity						
Any change (n=1975)	0.80 (0.62-1.03)	0.79 (0.61-1.02)	0.79 (0.61-1.03)	1.09 (0.82-1.45)	1.09 (0.81-1.46)	1.07 (0.80-1.43)
Change in cycle length						
Any change (n=1255)	0.85 (0.67-1.08)	0.85 (0.66-1.08)	0.86 (0.67-1.10)	1.26 (0.96 -1.65)	1.28 (0.97-1.68)	1.26 (0.96-1.66)
Change in cycle length[c]						
Shorter (n=767)	0.82 (0.62-1.09)	0.81 (0.61-1.08)	0.82 (0.61-1.09)	1.13 (0.84-1.53)	1.16 (0.86-1.58)	1.15 (0.84-1.56)
Longer (n=488)	0.90 (0.65-1.25)	0.90 (0.64-1.26)	0.92 (0.66-1.29)	1.55 (1.03-2.34)	1.53 (1.01-2.32)	1.52 (1.00-2.31)
Change in cycle length[d]						
Longer (n=488)	0.91 (0.65-1.26)	0.90 (0.64-1.26)	0.93 (0.66-1.30)	1.60 (1.06-2.41)	1.61 (1.05-2.45)	1.59 (1.05-2.43)
Change in cycle regularity						
Any change (n=1535)	0.84 (0.66-1.06)	0.83 (0.65-1.06)	0.83 (0.65-1.05)	0.85 (0.65-1.12)	0.85 (0.64-1.11)	0.83 (0.63-1.09)
Change in cycle regularity[c]						
More regular (n=626)	0.72 (0.52-1.00)	0.71 (0.51-0.99)	0.71 (0.51-0.98)	0.89 (0.64-1.25)	0.90 (0.64-1.26)	0.88 (0.62-1.24)
Less regular (n=909)	0.92 (0.69-1.21)	0.93 (0.70-1.23)	0.92 (0.69-1.22)	0.81 (0.59-1.11)	0.79 (0.57-1.09)	0.79 (0.57-1.08)

[a] Reference: uninfected
[b] Reference: unvaccinated
[c] Multinomial logistic model
[d] Excluding from analysis women who reported a change towards shorter length
Change in cycle characteristics defined by change in three categories: cycle regularity: i) very regular [+-3 days], ii) regular [within 5-7 days]; iii) usually irregular or always irregular; cycle length: i) <26 days; ii) 26-31 days; iii) ⩾32 days.
Model 1: adjusting for age at baseline, and follow up time
Model 2: model 1 + adjusting for BMI at end of follow up, weight change, race/ethnicity, education attainment, and region
Model 3: model 2 + mutually adjusting for SARS-CoV-2 infection and vaccination
742 hormonal contraception users at MOD2 were excluded from analysis

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Table S11. Odds Ratios (OR) and 95% confidence intervals (CI) of change in menstrual cycle characteristics in relation to SARS-CoV-2 infection and COVID-19 vaccination status, among participants who had regular (within 7 days) and normal cycle length (26-31 days) at baseline, the Nurses' Health Study 3 (NHS3), N=1,976

	SARS-CoV-2 infection[a] N=214			COVID-19 vaccination[b] N=1809		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
Change in usual menstrual characteristics	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Change in cycle length or regularity						
Any change (n=1261)	0.84 (0.62-1.14)	0.82 (0.61-1.11)	0.82 (0.61-1.11)	0.92 (0.66-1.30)	0.94 (0.66-1.33)	0.94 (0.66-1.32)
Change in cycle length						
Any change (n=685)	0.81 (0.59-1.11)	0.80 (0.58-1.09)	0.80 (0.58-1.10)	1.15 (0.81-1.64)	1.20 (0.83-1.72)	1.19 (0.83-1.71)
Change in cycle length[c]						
Shorter (n=419)	0.86 (0.59-1.23)	0.84 (0.58-1.21)	0.85 (0.58-1.22)	1.25 (0.82-1.90)	1.34 (0.87-2.06)	1.33 (0.87-2.05)
Longer (n=266)	0.72 (0.45-1.15)	0.69 (0.42-1.12)	0.68 (0.42-1.11)	1.01 (0.61-1.67)	0.97 (0.57-1.64)	0.95 (0.56-1.60)
Change in cycle length[d]						
Longer (n=266)	0.70 (0.43-1.12)	0.66 (0.41-1.08)	0.66 (0.41-1.08)	1.04 (0.63-1.74)	1.05 (0.62-1.79)	1.03 (0.60-1.75)
Change in cycle regularity						
Any change (n=1026)	0.91 (0.68-1.22)	0.90 (0.67-1.21)	0.89 (0.66-1.20)	0.79 (0.57-1.09)	0.78 (0.56-1.08)	0.77 (0.55-1.08)
Change in cycle regularity[c]						
More regular (n=264)	0.84 (0.54-1.33)	0.83 (0.52-1.31)	0.82 (0.52-1.30)	0.81 (0.51-1.30)	0.82 (0.51-1.34)	0.82 (0.51-1.34)
Less regular (n=762)	0.92 (0.66-1.27)	0.90 (0.65-1.26)	0.90 (0.64-1.25)	0.77 (0.53-1.11)	0.74 (0.51-1.08)	0.74 (0.51-1.08)

[a] Reference: uninfected
[b] Reference: unvaccinated
[c] Multinomial logistic model
[d] Excluding from analysis women who reported a change towards shorter length
Change in cycle characteristics defined by change in three categories: cycle regularity: i) very regular [+-3 days], ii) regular [within 5-7 days]; iii) usually irregular or always irregular or no periods; cycle length: i) <26 days; ii) 26-31 days; iii) ⩾32 days.
Model 1: adjusting for age at baseline, and follow up time
Model 2: model 1 + adjusting for BMI at end of follow up, weight change, race/ethnicity, education attainment, and region
Model 3: model 2 + mutually adjusting for SARS-CoV-2 infection and COVID-19 vaccination
742 hormonal contraception users at MOD2 were excluded from analysis

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Table S12. Odds Ratios (OR) and 95% confidence intervals (CI) of change in menstrual cycle characteristics in relation to SARS-CoV-2 infection and COVID-19 vaccination status, among participants who had irregular (>7 days) and short/long cycle length (<26 or ⩾32 days) at baseline, the Nurses' Health Study 3 (NHS3), N=1,140

	SARS-CoV-2 infection[a]			COVID-19 vaccination[b]		
	N=135			N=1026		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
Change in usual menstrual characteristics	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Change in cycle length or regularity						
Any change (n=966)	0.73 (0.46-1.17)	0.84 (0.52-1.34)	0.90 (0.56-1.46)	1.93 (1.21-3.09)	1.86 (1.14-3.03)	1.83 (1.12-3.00)
Change in cycle length						
Any change (n=723)	0.85 (0.59-1.23)	0.96 (0.66-1.40)	1.02 (0.70-1.50)	1.66 (1.12-2.45)	1.68 (1.12-2.52)	1.69 (1.12-2.54)
Change in cycle length[c]						
Shorter (n=439)	0.81 (0.53-1.23)	0.93 (0.61-1.44)	0.97 (0.62-1.50)	1.31 (0.86-1.99)	1.33 (0.86-2.06)	1.32 (0.85-2.06)
Longer (n=284)	0.91 (0.57-1.44)	1.00 (0.62-1.59)	1.10 (0.68-1.77)	2.77 (1.51-5.10)	2.79 (1.50-5.19)	2.82 (1.51-5.27)
Change in cycle length[d]						
Longer (n=284)	0.92 (0.58-1.45)	0.97 (0.61-1.56)	1.08 (0.67-1.75)	2.80 (1.52-5.15)	2.84 (1.53-5.28)	2.87 (1.54-5.35)
Change in cycle regularity						
Any change (n=709)	0.88 (0.61-1.28)	0.97 (0.66-1.41)	0.97 (0.66-1.42)	1.07 (0.72-1.60)	1.05 (0.70-1.59)	1.05 (0.69-1.59)
Change in cycle regularity[c]						
More regular (n=445)	0.76 (0.50-1.16)	0.83 (0.54-1.28)	0.83 (0.54-1.29)	1.09 (0.70-1.69)	1.08 (0.68-1.70)	1.05 (0.66-1.66)
Less regular (n=364)	1.10 (0.70-1.75)	1.22 (0.76-1.97)	1.22 (0.76-1.97)	1.02 (0.60-1.73)	0.98 (0.57-1.69)	1.01 (0.59-1.74)

[a] Reference: uninfected
[b] Reference: unvaccinated
[c] Multinomial logistic model
[d] Excluding from analysis women who reported a change towards shorter length
Change in cycle characteristics defined by change in three categories: cycle regularity: i) very regular [+-3 days], ii) regular [within 5-7 days]; iii) usually irregular or always irregular or no periods; cycle length: i) <26 days; ii) 26-31 days; iii) ⩾32 days.
Model 1: adjusting for age at baseline, and follow up time
Model 2: model 1 + adjusting for BMI at end of follow up, weight change, race/ethnicity, education attainment, and region
Model 3: model 2 + mutually adjusting for SARS-CoV-2 infection and COVID-19 vaccination
742 hormonal contraception users at MOD2 were excluded from analysis